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硕 士 学 位 论 文

钙及几种环境因子对拟南芥 (*Arabidopsis thaliana*) 钙受体 CAS 表达的影响

Effects of Calcium and Environmental Factors on CAS  
(Ca<sup>2+</sup>-sensing receptor) Expression in *Arabidopsis thaliana*

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## 摘 要

环境胁迫如酸雨、铝毒、镉毒、盐渍、干旱及温度等已严重危害植物的生长，进而影响到人类的生产生活。已有研究显示，外源钙的施加能缓解植物在这些环境胁迫下的伤害，但是钙受体（ $\text{Ca}^{2+}$ -sensing receptor, CAS）在外源钙缓解环境胁迫中发挥何种作用及变化规律却仍然不清楚。

本文以模式植物拟南芥（*Arabidopsis thaliana*）哥伦比亚野生型为材料，结合生理指标测定、蛋白质印迹（Western blot）等实验手段，研究了不同钙浓度在模拟酸雨、铝毒、镉毒、盐害、干旱、低温及高温胁迫下对拟南芥发芽率、根长及 CAS 的表达的影响。以期了解外源钙对这些胁迫所引起的植物生理生化的影响与 CAS 这个途径的可能关系可能，从而为钙缓解胁迫造成的植物伤害提供科学依据与技术支持，丰富外源钙缓解胁迫的机制。

以模式植物拟南芥野生型（WT）及 CAS 反义植株（CASas）为材料，结合生理指标测定、电镜观察、Western blot 及实时荧光定量 PCR 等实验手段，研究了生长于不同钙浓度下两种类型的拟南芥黄化苗在复绿过程中差异，以期了解钙及 CAS 在复绿过程中发挥的作用及钙促进复绿的机制。

主要取得以下初步的结果：

（1）酸雨、铝毒、镉毒及盐害作用下，均会造成拟南芥种子发芽率下降、胚根长度缩短及叶绿素流失，并且随着 pH 值降低，铝毒、镉毒及盐浓度的上升，上述生理指标下降得更为明显。高钙（20 mmol/L）生长下的拟南芥幼苗的种子发芽率、胚根长度及叶绿素含量均高于中钙（2 mmol/L）及低钙（0.2 mmol/L）生长下的拟南芥幼苗。表明钙能够缓解这些胁迫所造成的对拟南芥幼苗的伤害，在钙缺失的条件下，拟南芥幼苗更容易受到胁迫的伤害。

（2）通过 Western blot 的方法测定了萌发期及幼苗期拟南芥在不同钙及不同胁迫处理条件下的 CAS 表达变化。结果显示：拟南芥幼苗在模拟酸雨胁迫下，萌发期幼苗在高钙条件下 CAS 表达较为稳定，但在低钙作用下，CAS 的表达随着 pH 值下降而上升。幼苗期拟南芥幼苗的 CAS 表达随着处理天数的不同出现不同的表达规律，第一天，由于外源高钙的加入，高钙组的 CAS 表达出现瞬时

的高峰；第三天及第五天，高钙组的 CAS 表达较稳定，中钙及低钙的 CAS 表达由于胁迫的严重程度出较升高的情况；第七天，高钙组的 CAS 表达稳定且处于较高水平，中钙及低钙组处于低水平。拟南芥幼苗在重金属铝及镉毒胁迫下，萌发期及幼苗期条件下 CAS 的表达情况较为一致。高钙组幼苗的 CAS 表达情况最高，中钙组及低钙组幼苗 CAS 的表达较低，尤其是低钙组。拟南芥幼苗在盐害胁迫下，萌发期实验条件下，高钙能够提高 CAS 的表达，低钙条件 CAS 的表达水平较低。但在幼苗期实验条件下，低钙条件下的 CAS 表达水平随着盐浓度的上升而上升，而高钙组的 CAS 表达水平却下降，推测可能 20 mmol/L 的高钙加重了幼苗的盐害，20 mmol/L 的  $\text{CaCl}_2$  并不是缓解幼苗期实验条件下盐害的合适浓度。拟南芥幼苗在干旱胁迫时，幼苗期实验条件下外源钙能提高 CAS 的表达量，从而缓解干旱对拟南芥幼苗所造成的伤害。在低温条件下外源钙能提高 CAS 的表达量，但在高温的条件下，外源钙并没有明显增加 CAS 的表达量，但低钙组的 CAS 表达量明显低于中钙及高钙组。从以上结果中可以看出，钙及 CAS 信号系统很有可能参与了外源钙缓解这些胁迫的机制，调节的机制由于实验条件的不同，生长条件的不同，处理时间的不同出现不同的规律。

(3) 我们测定了生长于不同钙浓度条件下的拟南芥 WT 及 *CASas* 黄化苗在白光下复绿过程中的表型变化、叶绿素含量、叶绿体发育的差异，结果表明钙及 CAS 与拟南芥黄化苗复绿有一定的关系。高钙能够明显地促进复绿过程中拟南芥 WT 及 *CASas* 黄化苗的叶绿素含量及叶绿体发育，类囊体膜片层形成。实时荧光定量 PCR 及 Western blot 分析显示在光及外源钙加入时 CAS 的表达水平会上调。*CASas* 黄化苗的叶绿素含量少于及叶绿体发育迟于 WT 黄化苗。综合以上数据，我们认为高钙能促进复绿在一定程度上受到 CAS 表达水平的影响。

总之，外源钙能较好地缓解外界环境胁迫对拟南芥幼苗所造成的伤害，关于钙缓解这些胁迫的机制有很多，但钙及 CAS 信号系统很可能参与了缓解机制。而且钙能促进拟南芥黄化苗的复绿，并且很大程度上依赖于 CAS 的表达水平。

**关键词：**环境胁迫，钙，拟南芥，CAS，*CASas*，复绿

## Abstract

Environmental stresses, such as acid rain, aluminium toxicity, cadmium toxicity, saline, drought, low and high temperature have already been proved to severely limit the plant growth and productivity formation in individual and the biospheric scale. A numbers of studies have also shown that exogenous  $\text{Ca}^{2+}$  alleviates the plant injuries caused by mentioned environmental stresses. However, the roles of CAS ( $\text{Ca}^{2+}$ -sensing receptor), as a significant  $\text{Ca}^{2+}$  sensor, is not known in the plant responses to environmental stresses.

In this paper, in order to better understand the effects of different  $\text{Ca}^{2+}$  concentrations and environment stresses on plant metabolism and further elucidate the role of  $\text{Ca}^{2+}$ -CAS signal system in exogenous  $\text{Ca}^{2+}$  alleviated injuries, some physiological indicators measurements and western blot analysis were used for investigating the effects of different  $\text{Ca}^{2+}$  concentrations on *Arabidopsis thaliana* wild type (WT) seed germination, root length and CAS expression under various environmental stresses (acid rain, aluminium toxicity, cadmium toxicity, saline, drought, low and high temperatures). Moreover, in order to study the role of  $\text{Ca}^{2+}$  and CAS during the de-etiolation, we also studied the effects of different  $\text{Ca}^{2+}$  level on *Arabidopsis thaliana* WT and *CAS*s etiolated seedling during de-etiolation by the analysis of physiological indicators, electron microscope, western blot and real-time fluorescence quantitative PCR. The preliminary results are shown as follows.

(1) The seed germination rate, root length and chlorophyll content of *Arabidopsis thaliana* seedlings were significantly inhibited by acid rain, aluminium, cadmium and salt treatments. The inhibition was aggravated with the decrease of pH (simulated acid rain) and the increase of aluminium, cadmium and salt concentration.  $\text{Ca}^{2+}$  plays an important role in seed germination, root length and chlorophyll content. Compared with the control (2 mmol/L  $\text{CaCl}_2$ ), the 20 mmol/L  $\text{CaCl}_2$  treated *Arabidopsis thaliana* seedlings showed a higher germination rate, longer radical length and higher chlorophyll content, indicating that  $\text{Ca}^{2+}$  could alleviate the injuries caused by acid rain, aluminium, cadmium or salt stress. However, with low  $\text{Ca}^{2+}$  treatment, the injuries of *Arabidopsis thaliana* seedlings were increased with the intensified stresses.



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